QSLA2, a Chimeric Lysin with High Antipneumococcal Activity

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ABSTRACT:

Phage endolysins and bacterial autolysins represent a novel class of antibacterials (enzybiotics) against Gram-positive bacteria due to their unique ability to cleave the peptidoglycan in a generally species-specific manner. They provide a novel mode of action, kill fast and selectively pathogenic bacteria and show low toxicity or propensity to develop resistances. The Skl endolysin is encoded by the ΦSK137 bacteriophage of *Streptococcus mitis*, a human pathogen closely related to *Streptococcus pneumoniae*. It comprises an N-terminal catalytic module (CHAP amidase) and a C-terminal choline-binding module (CBM) closely related to that of the LytA amidase, the major pneumococcal autolysin. Systematic in-depth characterization of Skl and LytA have allowed the construction of two functional chimeric lysins by module swapping. By doing so, we have produced a new enzybiotic, QSLA2, that largely overcomes the antibacterial activity of LytA. By contrary, neither the chimera QLAS1 nor Skl efficiently lyse the pneumococcus from the outside, in spite of their capability to degrade purified cell walls. This variability in antimicrobial activity seems to be linked to *i*) the distinct affinity for choline and choline-induced dimerization showed by the CBMs of the parental enzymes, *ii*) the intrinsic efficiencies of the catalytic modules, *iii*) restraints imposed by the complex structural organization of intact cell walls and *iv*) the precise orientation of the two modules in the overall structure. Taken together, our results provide new clues on the role played by the CBM and module combination in the antibactericidal potential of pneumococcal lysins that can be used to improve or finely tune their activities.

Keywords: *Streptococcus pneumoniae*, enzybiotics, Skl, LytA, chimeric lysins.

1. Introduction

Cell wall lytic enzymes encoded by bacteriophages and bacteria constitute a novel and promising class of antimicrobials (enzybiotics). They usually consist of at least one catalytic module linked to other modules commonly involved in cell wall attachment and responsible for their stringent substrate specificity [1,2]. With the single exception of Cpl-7 endolysin, in the pneumococcal lytic enzymes this function relies on the choline-binding modules (CBMs) that specifically recognize the choline moieties attached to teichoic and lipoteichoic acids [3].

The effectiveness as antimicrobials of LytA, the major pneumococcal autolysin, and of endolysins Cpl-1 and Pal has been already demonstrated using different animal models of pneumococcal infection [2]. Nevertheless, identification of new lysins with high antipneumococcal activity is of primary interest. In this context, the Skl endolysin encoded by the ΦSK137 bacteriophage of *Streptococcus mitis* constituted a good candidate to be tested, as it degrades pneumococcal cell walls and comprises a CBM 67% identical in sequence to the homologous region of LytA [4]. By contrary they have unrelated catalytic modules (CM) with *N*-acetylmuramoyl-L-alanine amidase activities (CHAP and Amidase_2 respectively).

We have characterized the Skl endolysin in comparison with LytA, examining the affinity for choline and the changes promoted in structure and stability by choline recognition. Aiming to improve Skl activity and also to test how the modules of these two amidases conditioned their antimicrobial activity, we have created two chimeric lysins (QLAS1 and QSLA2), by module swapping, whose structural and functional features have been also investigated, including the *in vitro* antipneumococcal activity.

2. Experimental Section

2.1. Proteins

Skl and LytA were produced and purified as previously described [4-5]. Chimeric lysins were built from the genes of the parental enzymes, cloned in a pT7-7 plasmid, using the procedure of Wurch et al. [6]. The final constructions were overexpressed in *Escherichia coli* BL21 (DE3) and purified using the procedure used for Skl and LytA. All protein concentrations were determined spectrophotometrically using the theoretical extinction coefficients.

2.2. Methods

Choline titration curves of lysins were obtained by circular dichroism (DC), following the change in ellipticity promoted by choline binding in the far- and near-UV regions of the spectrum. The association state and its dependence on choline binding was analyzed by size-exclusion chromatography and ultracentrifugation, whereas the structural stability and modular organization were evaluated by thermal denaturation experiments using DC [5]. *In vitro* killing assays were carried out with different pneumococcal strains. Cells were resuspended in PBS, pH 6.8, to an $OD_{550nm} \cong 0.6$ and incubated at 37ºC for 60 min with or without the lytic enzymes, added at varying concentrations. Evolution of the OD_{550nm} was followed at different times and viable cells were measured after 15 and 60 min incubation. The CLSI protocol [7] was used to determine minimal inhibitory concentrations (MIC).

3. Results

Titration experiments showed that saturation of choline-binding sites and choline-induced dimerization of Skl occurs at ligand concentrations 20-times higher than those required by LytA. This finding could explain their different activities on purified cell walls [4], as full activity of pneumococcal lysins depends on the interaction with this aminoalcohol, and dimerization increased around 10-fold LytA activity [5]. To test this hypothesis, and taking advantage of the modular architecture of both lysins and of the similarity of sequences shown by their CBMs, two chimeric lysins were constructed by module interchange: QLAS1 (CM of LytA fused to the CBM of Skl) and QSLA2 (CM of Skl fused to the CBM of LytA). The structural and functional features of both chimeras were similar but not equals to those displayed by their respective modules in the parental enzymes, and both degraded isolated cell walls. Very different results were obtained however in the *in vitro* killing assays.

As shown in *Fig. 1*, only LytA and QSLA2, which carried the same CBM, behaved as efficient antimicrobials against pneumococcus. By contrary neither Skl nor QLAS1 reduced appreciably the number of viable cells under identical conditions. Of note, QSLA2 largely overcomes the antimicrobial activity of LytA considering both MIC values and cell viability after 15 or 60 min incubation with the lysins.

Taken together all these results provide a valuable information on the influence that each module has on the overall stability of these lysins and their activities than can be applied to modify pre-existing lysins or to create new ones.